

## Fatal Interactions: Fas-Induced Apoptosis of Mature T Cells

### Minireview

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Both immature cortical thymocytes and mature peripheral T cells may be induced to undergo apoptosis by ligation of the T cell antigen receptor (TCR). Intrathymic deletion is clearly an important mechanism by which the T cell system is rendered tolerant to many self-antigens, while the function of peripheral deletion is less clear. One possibility is that peripheral deletion is the mechanism that brings immune responses to a close after antigen has been cleared; another is that peripheral deletion is observed only with doses of antigen that are supraoptimal for inducing an effective T cell response; a third is that self-reactive T cells that evade clonal deletion in the thymus are subject to activation-induced deletion to maintain self-tolerance in the periphery. Peripheral T cell deletion could serve several or all of these functions.

Distinct apoptosis mechanisms act during the deletion of immature and mature T cells. Overexpression of *bcl-2* in transgenic mice has little effect on intrathymic clonal deletion, but inhibits superantigen-induced peripheral T cell deletion (Sentman et al., 1991; Strasser et al., 1991). Now Singer and Abbas (1994 [this issue of *Immunity*]) have shown a clear difference between intrathymic and peripheral T cell deletion in the requirement for the cell surface molecule Fas, also known as APO-1 or CD95. In this paper, a T cell receptor transgenic mouse line has been crossed with the *lpr* mutant, which does not express Fas due to a mutation in which insertion of a transposon disrupts the Fas gene (Watanabe-Fukunaga et al., 1992a). Injection of the specific antigenic peptide results in intrathymic and peripheral T cell deletion in TCR transgenic, but otherwise normal, animals; whereas in TCR transgenic *lpr/lpr* homozygotes there is intrathymic but not peripheral deletion.

This experiment knits together a number of previous studies in which different aspects of T cell deletion were characterized in *lpr* mutant mice. Several groups have analyzed the endogenous superantigen-induced intrathymic clonal deletion of T cells expressing TCR  $\text{V}\beta$  genes; in general, this form of deletion is not compromised by the *lpr* mutation (Kotzin et al., 1988; Singer et al., 1989), although there is a clonal deletion defect due to genetic background abnormalities in MRL-*lpr* mice (Smyth et al., 1992). In *lpr*-homozygous TCR transgenic mice that expressed the anti-HY/D<sup>+</sup> TCR on CD8<sup>+</sup> cells, male mice were able to delete the self-reactive thymocytes despite the lack of Fas (Zhou et al., 1991). In contrast, while injection of the exogenous superantigen staphylococcal enterotoxin B into normal mice results in transient T cell expansion followed by massive peripheral deletion, the same procedure in *lpr* mutant mice lead to a sustained expansion without deletion (Scott et al., 1993). The Singer and Abbas paper shows that these differences are not contingent on the antigenic system, since both thy-

mic deletion and failure to delete mature T cells occur in the same animals with the same peptide antigen.

The irrelevance of Fas in intrathymic clonal deletion is surprising, since among normal tissues the thymus expresses the highest level of Fas mRNA (Watanabe-Fukunaga et al., 1992b). One possibility is that Fas is indeed important in the thymus, but not as an agent of clonal deletion. Many thymocytes die because they fail to express a TCR with sufficient affinity for thymic major histocompatibility complex (MHC) class I or class II molecules. These cells unresponsive to thymic positive selection have been proposed to die "of neglect" (von Boehmer, 1986), but it is possible that this neglect, which consists of the failure to receive a survival and maturation signal through the TCR, allows them to receive an apoptotic signal through the Fas molecule. One could imagine two categories of neglected cells: those in which gene rearrangement fails to generate a TCR $\alpha\beta$ , and those in which the TCR, once generated, has insignificant affinity for MHC ligands present in the thymus. Experiments in anti-HY/D<sup>+</sup>-reactive TCR $\alpha\beta$  transgenic (+/+) versus *lpr* mice suggest that once an  $\alpha\beta$  TCR has been generated, positive selection acts normally, even in Fas-deficient thymocytes (Zhou et al., 1991; Sidman et al., 1992), although there is no consensus as regards the subsequent fate of these cells. While the generation of TCR-positive thymocytes is increased in *lpr* mice, this increase is abrogated by the presence of a TCR $\beta$  transgene (Zhou et al., 1993). From this, one might argue that any role for Fas in the thymus must be in TCR-negative cells prior to  $\beta$  locus rearrangement. Possibly Fas-mediated apoptosis removes thymocytes that fail to make an in-frame  $\beta$  rearrangement. A direct test of this idea would be to cross *lpr* with TCR $\beta$ -deficient mice. Such mice should not exhibit the early developmental arrest seen in TCR $\beta$ -deficient but otherwise normal mice (Monbaert et al., 1992); instead, immature cortical-type thymocytes should accumulate. Such an experiment must be in the works, but it would take courage to bet on the result.

It is difficult to postulate an important function for Fas in thymocyte maturation for another reason: the thymus does not express the known Fas ligand (FasL). This molecule, homologous to TNF $\alpha$  and TNF $\beta$  (LT $\alpha$ ), was recently identified by its ability to bind to a soluble form of Fas (Suda et al., 1993). FasL is likely to be the only important ligand of Fas, since the *gld* mutation, which causes a phenotype essentially identical to *lpr*, is due to a point mutation in the FasL gene (Takahashi et al., 1994). The abnormal phenotype consists of a multisystem autoimmune disease accompanied by the accumulation of huge numbers of functionally incompetent, CD4/CD8 negative, TCR $\alpha\beta$ -expressing T cells (Davidson et al., 1986). These cells have, at times, been attributed to an abnormal T cell maturation pathway that exists side-by-side with normal development in *lpr* mice; to the expansion of an extrathymic T cell development pathway; and to failure to destroy thymocytes rejected by intrathymic selection processes (Budd et al., 1987; Ohteki et al., 1990, 1992; Singer and Theofilopoulos, 1990). But it now

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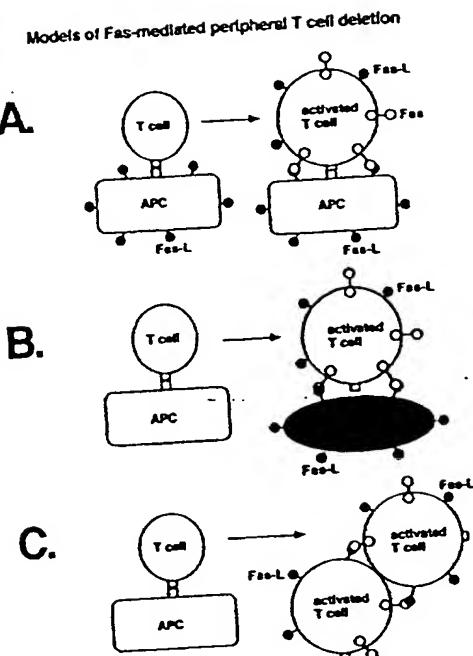


Figure 1. Models of Fas-Mediated Peripheral T Cell Deletion  
 (A) "Decadence," in which APCs constitutively express the FasL, but it is only after activation and differentiation that T cells express Fas and are susceptible to destruction as a consequence of repeat stimulation.  
 (B) "Nemesis," in which Fas-positive activated T cells undergo deletion only when they encounter FasL-positive cells specialized to induce their apoptosis.  
 (C) "Fraticide," in which activated T cells express both Fas and FasL, and induce apoptosis in one another.

seems fairly clear that at least most of them represent an end stage of peripheral T cell deletion, and that they accumulate because a signal through Fas is required for their removal in normal mice (Crispe and Huang, 1994).

Fas-dependent peripheral deletion of activated mature T cells implies that these T cells are susceptible to apoptosis induced by Fas ligation, and that they encounter the FasL. Susceptibility to Fas-induced apoptosis is not simply a function of cell surface Fas expression, since T cells show dramatic induction of Fas mRNA and cell surface protein within 2-3 days of activation, but treatment with cross-linking anti-Fas antibody does not cause their apoptosis until several days later (Miyawaki et al., 1992). Perhaps this is because the level of the anti-apoptosis protein Bcl-2 is high for the first few days after T cell activation, but falls thereafter (Akbar et al., 1993). To incorporate this idea into a model of peripheral deletion, one could propose that antigen-presenting cells (APCs) constitutively express the FasL, but resting T cells do not express Fas and, hence, their TCR ligation results in activation and proliferation. In contrast, already activated T cells express Fas molecules, and a subsequent encounter with an APC results in delivery of an

apoptotic signal. This model, illustrated in Figure 1A, is termed "Decadence." The idea is that the full flowering of the differentiation program of a T cell necessarily renders it susceptible to signals that lead to its destruction. The decadence model can readily explain the autoimmunity associated with Fas deficiency in the *lpr* mutant. Like Dorian Gray, any self-reactive T cells in *lpr* mice would have an extended lifespan, remain immune to the destructive sequelae of their actions, and continue to inflict unchecked damage on innocent bystanders.

An alternative model, illustrated in Figure 1B, is termed "Nemesis." In this model, activated peripheral T cells undergo apoptosis as a result of a unique cell interaction with FasL-positive cells. These cells are not the same as conventional APCs, and antigen recognition may or may not be essential for the interaction. But the nemesis cells do not simply correspond to all the non-APCs in the body, since FasL expression is restricted to a few tissues (Suda et al., 1993). The highest FasL RNA levels have been found in small intestine and testis, with significant amounts also in the lung, a very different tissue distribution from that of Fas, which is expressed at a high level in thymus, ovary, heart, and liver (Watanabe-Fukunaga et al., 1992b). The expression of FasL in small intestine is fascinating, since this is a major site for the homing of activated T cells (Sprent, 1976), and could therefore be a site at which they are programmed for deletion.

But the highest level of FasL expression in lymphoid tissue occurs in activated lymphocytes (Suda et al., 1993). In fact, activated spleen cells are the only tissue that expresses high levels of mRNA for both Fas and FasL. This suggests the model illustrated in Figure 1C: "Fraticide." In this model, activated T cells express both Fas and FasL, and induce apoptosis in one another. A corollary of this is that T cell responses with the highest precursor frequency will generate abundant fratricidal FasL-positive T cells, while responses with a much lower starting frequency of T cells would generate many fewer. In the primary response of a normal mouse to an exogenous antigen, the probability of an encounter between two Fas/FasL-positive cells would therefore be low, allowing clonal expansion to take place. The migration of T cells out of lymph nodes, several days after antigenic stimulation, would serve to spread out the activated clones and protect further against fratricidal interactions. But this mechanism could limit further clonal expansion in subsequent restimulation events, preventing dominance of the immune system by a few expanded specificities.

The clearest evidence for activation-driven peripheral T cell deletion comes from studies with normal T cells responding to superantigens (Kawabe and Ochi, 1991; Webb et al., 1990; Huang and Crispe, 1993), and in TCR transgenic mice responding to infusions of antigenic cells or antigenic peptide (Rocha and von Boehmer, 1992; Mamalaki et al., 1993). Both of these systems offer the huge experimental advantage that the responding cells express a unique TCR or set of TCRs, and are abundant enough that they can be observed directly; but these benefits come with a price. Knowledge of T cell physiology derived from these models is strongly biased toward phenomena that occur in very

abundant populations of T cells. Experiments in such systems have shown either transient T cell expansion followed by deletion, or peripheral deletion as the only detectable response. We are left to wonder whether physiologically important immune phenomena, such as the maturation of memory T cells, will occur in the claustrophobic environment of a mouse where every T cell expresses the same antigen receptor.

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